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STUDIES IN THE PHYSIOLOGY OF THE FUNGI

X. GERMINATION OF THE SPORES OF CERTAIN FUNGI IN RELATION TO HYDROGEN ION CONCENTRATION¹

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INTRODUCTION

The hydrogen ion concentration of culture media or solutions has come to be regarded in recent years as one of the most important factors influencing physiological phenomena. A voluminous literature is found dealing with the toxic properties of H and OH ions in a general way, but the earlier investigators, like many of the later ones, were handicapped by lack of methods, or experience with methods, for the direct determination of hydrogen ion concentration. With such limitation in technique, conductivity data have frequently been employed in the interpretations made. This method is, however, inapplicable when other solutes are introduced, and the presence of strong buffers, whether inorganic or organic, would render most difficult any computation of active acidity or alkalinity.

Some of the questions which are unanswered are: What is the effect of hydrogen ion concentration upon the rate of germination of the spores of certain fungi, or, what is the range within which the most favorable germination occurs? Such questions suggested the desirability of conducting the investigation reported in this paper, and the scarcity of definite literature dealing with this particular phase has been one of the greatest incentives to the pursuance of the problem.

REVIEW OF LITERATURE

Clark ('99) seems to have been one of the pioneer workers on the toxicity of acids, alkalis, oxidizing agents, and salts of the heavier metals, towards the growth of certain fungi. Using the

¹An investigation carried out at the Missouri Botanical Garden in the Graduate Laboratory of the Henry Shaw School of Botany of Washington University, and submitted as a thesis, in partial fulfillment of the requirements for the degree of master of arts in the Henry Shaw School of Botany of Washington University.

hanging-drop method, he determined, in a nutrient medium, approximately the relative and absolute toxic properties of many deleterious agents as shown by their influence on spore germination, mycelial development, and fructification. The medium used throughout this study was an infusion of sugar beet, as experiments have shown this to be the most suitable and satisfactory medium for all the forms. The toxicity of the various acids and alkalis towards moulds is shown in detail, and interest centers upon (1) the average inhibiting concentration for germination and development, and (2) the average killing concentration, the organisms employed being *Aspergillus flavus*, *Sterigmatocystis nigra*, *Oedocephalum albidum*, *Penicillium glaucum*, and *Botrytis vulgaris*.

It was a general rule with all acids and alkalis that when the concentrations were not sufficient to cause distinct injury, stimulation of growth followed the slight retardation, such cultures taking on new vigor and surpassing the controls. Spores of *Botrytis* were most easily killed, while those of *Penicillium* offered the greatest resistance. Comparing the results with conductivity data, Clark concludes that the OH ion is more toxic towards fungi than the H ion. It is to be remembered, however, that the hydrogen ion concentration of none of his solutions was known. Nevertheless, this is of special interest when taken in conjunction with data which I shall present later.

Using distilled water as a medium, Stevens ('98) studied the effect of salts, bases, and acids upon the germination of the following fungous spores: *Botrytis vulgaris*, *Macrosporium* sp., *Penicillium crustaceum*, *Gloeosporium musarum*, and *Uromyces caryophyllinus*. As might be expected, germination of most of these species is not perfect in distilled water or else there is considerable variability in the results. Abnormal and distorted mycelium appear more frequently in the acid solution than in ordinary nutrient media. With *Macrosporium* and *Penicillium* neither HCl nor H₂SO₄ prevented growth, and the behavior of *Uromyces* towards these acids was quite variable. Further work with alkalis tends to indicate that KOH, NaOH, and NH₄OH have a low toxic value. *Penicillium* generally offered the greatest resistance to the different agents. From the data obtained,

the author concludes that various fungi exhibit varying degrees of resistance to poisons, and that the limits of resistance may vary in the species. Even though little data were at hand, Stevens concluded that the spores of fungi, when compared to the roots of seedlings, were less susceptible to toxic action.

Duggar ('01) made an extensive study of spore germination, including certain chemical as well as physical stimuli. Using distilled water as the medium, he found that organic acids stimulated germination but the percentage of germination was not great. The stimulus of N/100 or less of acetic acid to *Aspergillus flavus* and *Sterigmatocystis nigra* was very noticeable. Oxalic acid was more pronouncedly stimulating with *Sterigmatocystis*, N/100 producing maximum germination, whereas this concentration totally inhibited germination of *Aspergillus*. In this work, the considerable extent of individual variation was emphasized.

Ferguson ('02), using an artificial digestive fluid containing solutions of pepsin in distilled water combined with different amounts of HCl also in distilled water, studied the germination of spores of *Agaricus campestris*, *Coprinus comatus*, and *Calvatia cyathiforme*, but germination was so erratic that she was unable to draw any definite conclusions.

Brooks ('06) studied the effect of temperature on the toxic properties of CuSO_4 , HNO_3 , and H_2SO_4 , as shown by the effect of these substances on the germination and growth of certain fungi. In all the experiments, beet decoction was used as the nutrient medium, the stock infusion containing 600 gms. of beets per 1000 cc. of water. At the time of using, the decoction was diluted by the addition of the toxic substance and water to one-half of its former nutritive value. Usually, above the provisional optimum, the deleterious action of the toxic agents increased very rapidly with rise in temperature. Spores inhibited by cold were not greatly injured when exposed to harmful agents. In all instances, however, the injurious effects were least at the optimum temperature for the fungus; however, the effects of the three chemicals were very different.

Ayers ('16), making cultures of streptococci in a broth containing 1 per cent cerevisine, 1 per cent peptone, 1 per cent test

substance (glucose, lactose, etc.), and distilled water, obtained data indicating that streptococci reach more or less definite hydrogen ion concentration and that there are two limiting zones, P_H 4.6–4.8 and P_H 5.5–6.0. A very large percentage of the streptococci from cases of human infection reached only the lower limit of hydrogen ion concentration, a fact that is very striking.

Morgan and Gruzit ('16) found that soil solutions adjusted to various reactions by N/100 mineral acid and N/100 alkali, when mixed with sterile quartz, showed variations in the type and number of bacteria. A solution with N/1000 alkali gave the best growth while N/1200 acid exerted marked toxicity. In alkaline solutions the number of bacteria increased up to the point of faint alkalinity and then decreased after passing this point; whereas in acid solutions the number of bacteria increased with decrease in acidity.

Zeller ('16) found that the reaction of the medium was a most important factor influencing the growth and metabolism of *Lenzites saepiaria*. A medium of Thaxter's glucose-potato-hard agar possessing the faintest alkalinity failed to produce the slightest growth, but, on being readjusted to slight acidity, it gave good growth. Spaulding ('11) found that the same organism was unusually sensitive to alkaline media, and obtained luxuriant growth with one-fourth of 1 per cent H_2SO_4 . Other investigators have published similar results.

Salter ('16) found that the reproduction of legume bacteria in Ashby's mannite solution and in a soil solution was greatly influenced by the reaction of the medium. A neutral or slightly acid reaction in mannite solution, the means of determination of which are not stated, proved to be the most favorable for the production of the red clover organism. Inhibition of growth was evident in slightly alkaline solutions, and no growth was found in the presence of 1 per cent normal alkali. *Bacillus radicumicola* from alfalfa, on the other hand, exhibited great sensitiveness towards acidity, retardation of growth being noticed with .5 per cent normal H_2SO_4 . The organism grew best in faintly alkaline or neutral mannite solution.

Clark and Lubs ('17) grew *Aspergillus niger* on a medium con-

sisting of 1 gm. KH_2PO_4 , 3 gms. NaNO_3 , .5 gm. MgSO_4 , 100 gms. sucrose in 1 liter water, and on the seventh day found the hydrogen ion concentration to be 2×10^{-2} . They comment upon Waterman's estimate that the critical limit for *Penicillium glaucum* is about 1×10^{-5} N. H. and for *Aspergillus niger* about 4.5×10^{-5} N. H. The reviewers think that the only explanation for such discordant results must lie in a confusion in the method of expressing hydrogen ion concentration.

Fred and Loomis ('17) found that a mannitol solution with a neutral reaction gave the highest count of *B. radiculicola* from alfalfa. The addition of small amounts of alkali did not appreciably alter the number of bacteria; however, acid in equivalent amounts either retarded or inhibited growth. From the curve of hydrogen ion concentration, they are inclined to think that the apparent resistance of the legume bacteria to alkali is due to the slight concentration of hydroxyl ions in the mannitol solution. This work confirms that of Salter.

Gruzit ('17) studied the effect of acids and alkalis on soil bacteria in soil solution, and found that soil bacteria were extremely sensitive to an acid reaction. H_2SO_4 at a concentration of N/1200 destroyed about 99.6 per cent of the bacteria; N/1400 killed about 93.0 per cent of the organisms; and N/2840 prevented the growth of about 43.0 per cent. On the other hand, N/1000 alkali gave the maximum number of bacteria.

Taylor ('17) determined the concentrations of a few organic and inorganic acids necessary to check the growth of various organisms. He obtained data which led him to conclude that there is a great variation or specificity in their activity toward different organisms.

Wolf and Harris ('17) observed that the acidity of the medium may either delay or entirely stop the growth of *B. perfringens* and *B. sporogenes*, the critical concentration of the former being P_H 4.82 and the latter, P_H 4.94. All the acids tested gave very similar effects and showed practically no specific qualities.

Wright ('17) studied the importance of uniform culture media, and obtained data which clearly emphasize the many discrepancies that exist when the culture medium is adjusted by means of phenolphthalein titration. He found that the hydrogen ion

concentration of the culture medium and the resistance of organisms to the action of disinfectants afford a definite relation, the greatest resistance being obtained with a culture medium having a hydrogen ion concentration P_H 6.0–7.0.

Fred and Davenport ('18) found that the growth of the nitrogen-assimilating bacteria in culture solutions of different reactions was related to the hydrogen ion concentration of the medium. Of the legume bacteria, the organisms of alfalfa were the most sensitive to hydrogen ion concentration, the limit of growth on the acid side being between P_H 5.4 and 5.6; while, on the contrary, the organisms of lupine were the most resistant, the limit of growth on the acid side being P_H 4.6. Sodium hydroxide did not cause any noticeable toxicity towards the legume bacteria until added in greater quantities than N/125 and appeared to have only one-tenth the toxic properties of H_2SO_4 towards these organisms. The authors cite Beijerinck as having secured optimum growth of *Rhizobium leguminosarum* in N/166.6 acid, but explain the disagreement of results on the ground of employing different culture media. *Azotobacter* proved to be very sensitive to slight changes of reaction and was able to grow only within the narrow limits, P_H 6.5–8.6.

Meacham ('18) determined the hydrogen ion concentration of synthetic and malt-extract media necessary to inhibit the growth of *Lenzites saepiarum*, *Fomes rosens*, *Coniophora cerebella*, and *Merulius lacrymans*. Growth is not inhibited until a very high hydrogen ion concentration is reached, and, while the different fungi show considerable fluctuations, the organisms respond in much the same way. In general, growth proceeds in a straight line until about P_H 2.6; decreases almost abruptly at P_H 2.6, the range P_H 2.6–1.9 being termed the "critical range"; from P_H 1.9, the decline is more gradual and the limiting P_H value appears to be about 1.7. Prior to the sudden decrease at P_H 2.6, there frequently occurs a maximum of growth, usually about P_H 3.0.

Krönig and Paul ('97) found a solution of HNO_3 to be distinctly more toxic to anthrax spores than the same concentration of HCl . Their results with acetic acid were similar to those obtained by Clark ('99), but the results with alkalis were not consistent with Clark's.

METHODS

The methods employed in this investigation, as described by Clark ('99) and Duggar ('01), are substantially those used by others in this laboratory.

Organisms.—The fungi used were *Aspergillus niger*, *Penicillium cyclopium*, *Fusarium* sp., *Botrytis cinerea*, and *Lenzites saepiaria*. An attempt was made to use *Colletotrichum lindemuthianum*, but, owing to the failure to obtain germination in the control culture solutions, this organism was discarded. In the test-tube cultures from which the spores were obtained, the fungi were grown on potato agar made according to Duggar, Severy, and Schmitz ('17); i. e., 230 gms. of potato were cut into small pieces, autoclaved in 1 litre of water for 1 hr. at 15 lbs. pressure, filtered while hot, 15 gms. of agar added, the mixture then autoclaved for 15 minutes at 15 lbs., correction made for loss of water, and finally tubed, sterilized, and slanted. The cultures were allowed to grow at room temperature, and the spores were always taken from cultures that were from 10 to 15 days old.

Culture solutions.—The composition of the culture solutions was based primarily on Clark and Lubs's ('17) titration curve of ortho-phosphoric acid. Stock solutions of M/5 mannite in M/10 H_3PO_4 and M/5 mannite in N/5 NaOH were made. Into sterile Pyrex flasks, 100 cc. of the M/5 mannite-M/10 H_3PO_4 solution were placed, and increasing proportions of M/5 mannite-N/5 NaOH were added. The flasks were plugged with cotton and sterilized at 15 lbs. pressure for 15 minutes, after which the hydrogen ion concentrations were determined by the colorimetric method as outlined by Clark and Lubs ('17). The procedure was as follows:

To a test-tube containing a 10-cc. portion of the culture fluid the proper indicator was added, and the color developed compared with the colors obtained upon the addition of the same indicator to tubes containing equal quantities of standard buffer solutions. All solutions were made from the best chemicals, purified according to Clark and Lubs ('17), and made up with doubly distilled water. A series of solutions was thus obtained ranging in hydrogen ion concentration from P_H 2.8 to 10.0+. In nearly every case the determined value was identical with

the calculated value, the greatest divergence being .2. In the alkaline range the concentration of the OH ions in the last solution was beyond the range of the indicator, so it has been designated as 10.0+. The ten solutions, termed a series, are as follows: P_H 2.8, 3.1, 4.4, 5.0, 6.2, 7.0, 7.4, 8.8, 9.6, and 10.0+.

Small portions of each of the solutions were transferred to sterile test-tubes permanently labeled and fitted with rubber stoppers, through each of which passed a glass rod drawn to a blunt point. All transfers of a solution were made with its particular rod, thus avoiding all chances of mixing solutions. Fresh solutions were placed in the tubes from time to time, and verifications of hydrogen ion concentration frequently made.

Method of culture.—The method of culture was based primarily on the hanging-drop or Van Tieghem cell. The glass cylinders employed were perfectly ground at each end and measured 18.0 mm. in diameter and 9.0 mm. in height, possessing therefore a volume of 2.3 cc. The cylinders were cemented to the slide by means of wax; the tops of the cylinders were then coated with a thin ring of vaseline, and the cells completed by sealing cover glasses to the tops of the cylinders. A small nick was made in each ring of vaseline, prior to sealing, so that equilibrium of air pressure might exist when the cultures were placed in the incubator. About 15 minutes later, the cultures were examined and the cover glasses slightly pressed to the cylinders in order to insure a perfect sealing. Two cells were placed on each slide and labeled by gumming numbered and lettered labels to the center of the slip.

Four or five drops of the same solution as that to be used in the culture were placed in the bottom of the cell, the object being to establish a complete equilibrium of vapor pressures in the cells and to prevent changes in the concentration of the solution tested, as shown by Clark ('99). A few drops of the same solution were also placed on a sterile slide, and spores transferred from a pure culture to the slide by means of a sterile platinum needle. A solution of spores was thus made and thoroughly stirred in order to prevent the spores from adhering in bunches. A drop of the spore solution was transferred from the slide to the cover glass by means of a clean,

sterile, glass rod drawn to a small point. The cover glass bearing the culture was then inverted on the cell and gently pressed until completely closed with the exception of the minute opening previously described. All cultures were made up at room temperature, and, when a set of cultures was completed, all were placed in an incubator and kept at a constant temperature. Cultures of each organism were incubated at 22° C., 27° C., and 31° C., respectively, with the exception of *Lenzites saepiaria*.

Care of cells, etc.—The glass rings and slide composing the cells were never used a second time without being taken apart and thoroughly cleaned by boiling in alkali, soaking in cleansing mixture, and repeatedly rinsed in distilled water. They were then sterilized in an oven at 150° C. for 1 hour and protected from dust until needed. The cover glasses were treated similarly except that they were boiled longer in order to remove all traces of vaseline, and finally dipped in alcohol and wiped dry. All slides, rods, etc., were placed in water after having been used, and given the same general treatment before setting up another series.

Examination of cultures and data.—Cultures were made up, as a rule, in the afternoon and examined at different intervals, depending on the length of time required for the spores of a particular organism to germinate, as determined by a preliminary experiment. Spore counts were made from five fields of the hanging drop, and the average percentage of germination recorded. Where possible three different readings were made with each set of cultures, but often the mycelial growth was so luxuriant that only two were possible. All of the experiments were run in duplicate, and the germination data reported in this paper represent the percentage averages of the two cultures. Although slight fluctuations occurred throughout the work, the result from any cell agreed very closely with that of the duplicate.

Curves.—The curves are developed from the percentage averages, as indicated above, each curve representing the final reading of the germination quantities of a certain organism at a particular temperature. The percentages of spore germination are plotted as ordinates and the hydrogen ion concentration of

the solutions as abscissae. Curves corresponding to each of the temperatures of incubation are found in each figure, the solid line representing 22° C., the dotted line, 27° C., and the broken line, 31° C.

EXPERIMENTAL DATA

In examining the experimental results, it must be borne in mind that perfect germination is not to be expected with these fungi in a solution containing mannite as the sole nutrient. In fact, dextrose would perhaps have yielded higher germination percentages, but it is not certain that it would remain stable with the treatment given. Moreover, in this preliminary work it was not desired to employ a full nutrient solution on account of greater difficulties of P_H adjustment.

The influence of hydrogen ion concentration upon the germination of the spores of *Aspergillus niger* may be seen by referring to table I.

TABLE I

ASPERGILLUS NIGER. AVERAGE PERCENTAGES OF SPORE GERMINATION IN M/5 MANNITE AT DIFFERENT TEMPERATURES AND AT VARIOUS HYDROGEN ION CONCENTRATIONS

Temp.	Hrs.	Hydrogen ion concentration, P_H								
		2.8	3.1	4.4	5.0	6.2	7.0	7.4	8.8	9.6
22° C.	16	33.1	64.2	33.4	19.8	13.7	9.0	2.0	0.0	0.0
	26	63.5	83.1	55.0	42.1	18.1	13.4	14.0	8.2	0.0
	38	66.8	87.5	55.6	42.9	20.2	15.5	15.8	8.6	0.0
27° C.	16	45.3	69.4	25.9	20.0	5.5	15.1	0.0	0.0	0.0
	26	53.0	71.8	31.0	23.7	7.1	20.1	0.0	0.0	0.0
31° C.	16	22.6	32.6	46.6	44.0	24.4	18.6	0.0	0.0	0.0
	26	28.1	50.4	47.7	45.4	32.0	22.6	3.0	0.0	0.0

Incubated at 22° C., the data show that maximum germination is obtained in the culture having a hydrogen ion concentration of P_H 3.1. With further increase in hydrogen ion concentration, there is a marked inhibition of germination, the percentage decreasing from 64.2 at P_H 3.1 to 33.1 at P_H 2.8; while, with decrease in hydrogen ion concentration from P_H 3.1, there is a

general decrease in percentage of germination. Only a comparatively small amount of germination is obtained in the culture testing P_H 7.4 and no germination whatever is evident at P_H 8.8. The limiting concentration of hydrogen ions lies between P_H 7.4 and 8.8 on the alkaline side, and below P_H 2.8 on the acid side.

Examination of the same series after an incubation of 26 and 38 hours, respectively, shows that germination has increased in all the cultures and that there have been several slight changes in the curve of germination. A slight maximum is noticed on the alkaline side at P_H 7.4, and a relatively low percentage of germination is obtained at P_H 8.8, which at 16 hours was the limiting concentration.

A series incubated at 27° C. for 16 hours gives a general curve of germination very similar to that incubated at 22° C. with the exception of several slight shifts. A maximum of germination is obtained at P_H 3.1, as before, but the limiting concentration on the alkaline side shifts toward neutrality; thus even at P_H 7.4 there is total inhibition of germination, as compared with P_H 9.6 in the first series. With decrease in hydrogen ion concentration from P_H 3.1 there is a general decrease in percentage of germination to culture P_H 6.2 where a minimum is reached, followed by a relatively small rise at P_H 7.0. Upon incubating the series for 10 additional hours, the same relations of germination are obtained. On account of the luxuriant mycelial growth, only two readings were possible with this series.

Incubation at 31° C. gives slightly different results from those incubated at 27° C. At the time of the first reading the maximum count occurs at P_H 4.4, and there is no evidence of stimulation of germination in the neutral or slightly alkaline cultures. On the other hand, germination decreases with decrease in H ion concentration. from P_H 3.1 to 7.0, the limiting concentration proving to be P_H 7.4. In the final reading maximum germination on the acid side shifts to P_H 3.1, thus making the maximum germination at each temperature occur at P_H 3.1; and the remaining figures confirm the results of the previous examination.

These relations are shown graphically in the curves of fig. 1.

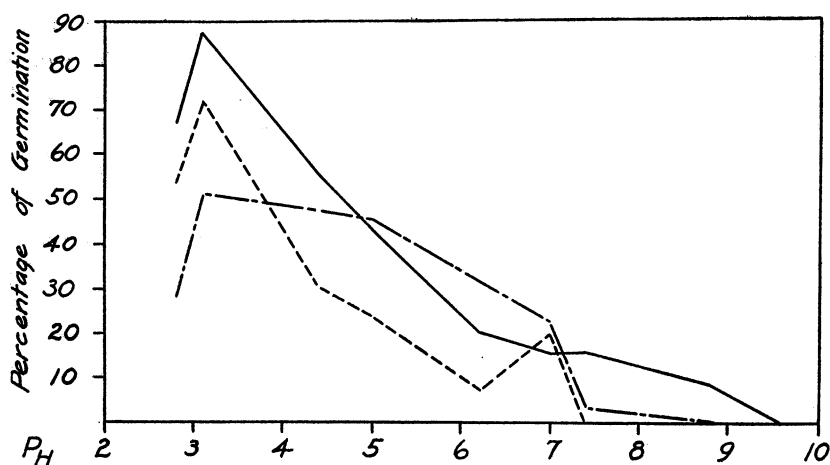


Fig. 1. *Aspergillus niger*. Graphic representation of the relation of germination to H ion concentration.

The data obtained with spores of *Penicillium cyclopium* as given in table II, are somewhat similar to those with *Aspergillus niger*.

TABLE II

PENICILLIUM CYCLOPIUM. AVERAGE PERCENTAGES OF SPORE GERMINATION IN M/5 MANNITE AT DIFFERENT TEMPERATURES AND AT VARIOUS HYDROGEN ION CONCENTRATIONS

Temp.	Hrs.	Hydrogen ion concentration, P _H									
		2.8	3.1	4.4	5.0	6.2	7.0	7.4	8.8	9.6	10.0+
22° C.	18	4.1	13.5	16.8	14.0	7.1	6.6	10.5	4.4	2.1	0.0
	27	10.3	21.1	30.7	16.6	10.9	8.1	28.7	10.5	2.7	0.0
	37	11.4	22.7	32.3	23.6	11.9	8.6	31.9	13.0	3.1	1.7
27° C.	18	32.2	35.5	22.9	20.7	10.5	12.3	7.0	0.0	0.0	0.0
	27	58.6	65.6	52.2	32.5	18.1	28.7	12.1	0.0	0.0	0.0
	37	59.8	66.6	55.4	35.1	22.0	33.2	18.7	0.0	0.0	0.0
31° C.	18	15.6	44.2	29.3	13.4	9.7	5.6	9.6	0.0	0.0	0.0
	27	30.0	57.8	39.3	25.2	19.5	6.8	12.1	5.0	0.0	0.0

When incubated at 22° C. for 18 hours, maximum germination is obtained in the culture with a hydrogen ion concentration of P_H 4.4, and percentage germination decreases with decrease in hydrogen ion concentration to the culture testing P_H 7.0 where

minimum germination is obtained. At P_H 7.4 there is a slight increase followed by a gradual decline to the culture possessing an exponent 9.6, the limiting concentration appearing to be beyond 10.0+. With increase of hydrogen ion concentration above P_H 4.4, there is a decrease in percentage of germination, but the limiting concentration evidently lies above the concentration P_H 2.8. Readings at incubation periods of 27 and 37 hours, respectively, give very similar results to those of the first reading, the only difference being that relatively slight germination is obtained in the culture with the value P_H 10.0+, which was formerly the limiting concentration. In no other case did *Penicillium* germinate at this relatively extreme alkalinity, and, inasmuch as these spores frequently collected in bunches, it is thought that the apparently erratic germination might be due to this fact.

At 27° C. maximum germination is obtained in the culture where the exponent is P_H 3.1, and the curve proceeds in the same general direction as before with the exception that minimum germination is obtained at P_H 6.2, as compared with P_H 7.0 in the former case. A slight rise is evident at P_H 7.0, only to be followed by a decline at P_H 7.4. On the acid side, the limiting concentration occurs below P_H 2.8, whereas on the alkaline side it occurs about P_H 8.8. Both examinations of prolonged incubation substantiate the data obtained from the first reading, the only difference being a gradual increase of germination with increase of incubation interval.

The same curve of germination is obtained at 31° C. The characteristic maximum occurs in the same culture as before, exhibiting the value P_H 3.1; germination decreases with decrease in hydrogen ion concentration to the culture testing P_H 7.0 where the minimum is obtained. A slight stimulation in germination is noted at P_H 7.4, but no germination whatever is noticed with further decrease in hydrogen ion concentration. With an additional incubation of nine hours at the same temperature, germination relations remain practically the same. The range of germination is extended to P_H 8.8, as compared with P_H 7.4 at the first examination, and germination is extremely low at P_H 7.0. Mycelial growth was so luxuriant in this series that it was

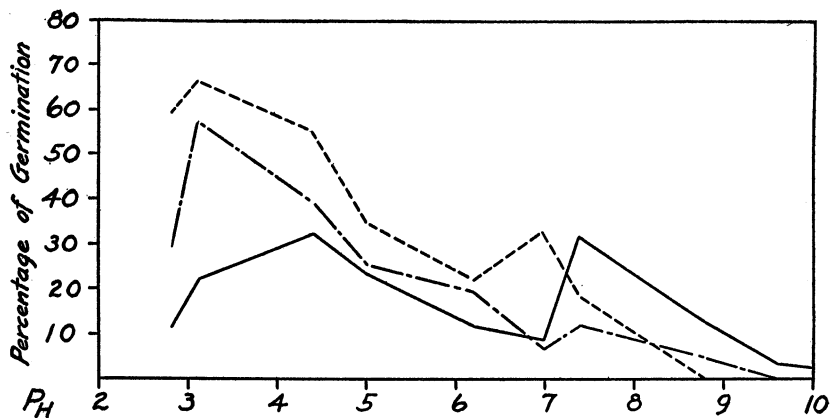


Fig. 2. *Penicillium cyclopium*. Graphic representation of the relation of germination to H ion concentration.

impossible to make a third reading. These relations are shown graphically in fig. 2.

An acid reaction decidedly favors spore germination of *Botrytis cinerea*, as seen by referring to table III.

TABLE III

BOTRYTIS CINEREA. AVERAGE PERCENTAGES OF SPORE GERMINATION IN M/5 MANNITE AT DIFFERENT TEMPERATURES AND AT VARIOUS HYDROGEN ION CONCENTRATIONS

Temp.	Hrs.	Hydrogen ion concentration, P _H									
		2.8	3.1	4.4	5.0	6.2	7.0	7.4	8.8	9.6	10.0+
22° C.	6	70.0	86.6	63.4	52.6	48.2	22.3	0.0	0.0	0.0	0.0
	21	77.1	90.2	72.9	62.6	57.8	46.0	0.0	0.0	0.0	0.0
27° C.	6	42.0	32.2	50.0	22.3	15.3	0.0	0.0	0.0	0.0	0.0
	21	92.4	66.4	61.2	34.9	30.1	0.0	0.0	0.0	0.0	0.0

Incubated at 22° C. no germination is obtained at P_H 7.4; little germination is obtained at P_H 7.0; and, with increasing hydrogen ion concentration, the germination quantities increase until a crest is reached in the culture with P_H 3.1. With further increase of hydrogen ion concentration, there is a diminution in percentage of germination. The data obtained after an incubation period of 21 hours are very consistent with those of the

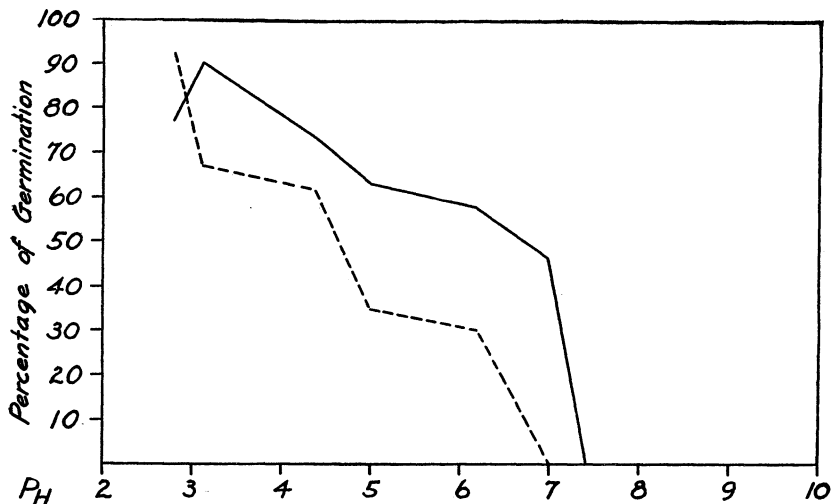


Fig. 3. *Botrytis cinerea*. Graphic representation of the relation of germination to H ion concentration.

6-hour period. Germination at 27° C. proved very similar to that at 22° C., except for the fact that the entire curve appears to have shifted one remove towards the acid side.

A series was made up and incubated at 31° C., but frequently the temperature went as high as 31.5° C. In no culture was there any sign of germination. This incidental datum is in accord with the results obtained by Duggar ('01). He found that a temperature of 32° C. was distinctly injurious to spores of *Botrytis*. Figure 3 exhibits the curves of germination at each of the successful temperatures.

TABLE IV

FUSARIUM SP. AVERAGE PERCENTAGES OF SPORE GERMINATION IN M/5 MANNITE AT DIFFERENT TEMPERATURES AND AT VARIOUS HYDROGEN ION CONCENTRATIONS

Temp.	Hrs.	Hydrogen ion concentration, P _H									
		2.8	3.1	4.4	5.0	6.2	7.0	7.4	8.8	9.6	10.0+
22° C.	6	0.0	0.0	7.2	9.5	11.6	11.7	65.3	21.3	8.5	24.6
	20	57.8	38.0	21.9	20.7	16.0	20.1	67.7	36.6	27.7	26.9
27° C.	6	0.0	9.7	31.0	45.4	18.0	23.3	50.0	23.7	5.1	0.0
	20	7.9	65.8	59.6	56.9	33.3	39.6	66.3	24.7	15.6	7.9

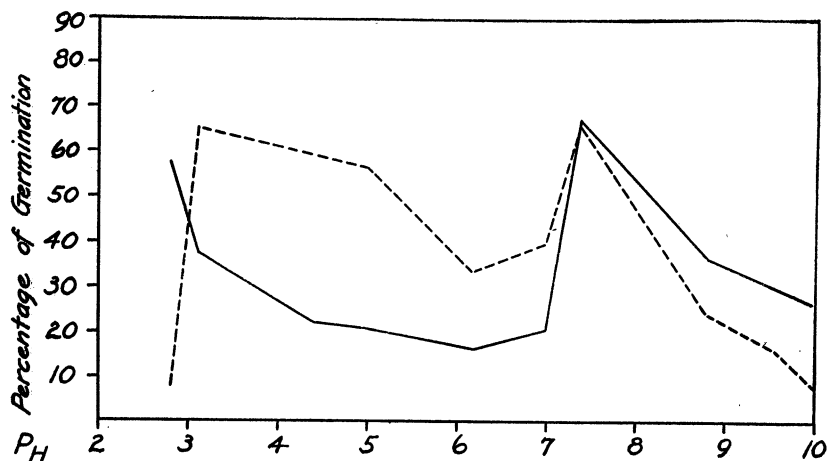


Fig. 4. *Fusarium* sp. Graphic representation of the relation of germination to H ion concentration.

Table IV shows that spores of *Fusarium* sp. are capable of germination over an extremely wide range of reaction. After incubation for 6 hours at 22° C. no germination is evident in the solution made to test P_H 2.8, and the same is true with the culture testing P_H 3.1, in very noticeable contrast with the results yielded by other forms.

Relatively small percentages of germination are obtained at P_H 4.4, and germination gradually increases with decrease in hydrogen ion concentration until a very pronounced maximum is reached at P_H 7.4. After this maximum, the curve declines only to rise suddenly at P_H 10.0+. Upon further incubation, germination progresses rapidly in the extreme acid cultures, that testing P_H 2.8 exhibiting maximum germination on the acid side. From this maximum, germination decreases to the culture possessing the exponent 6.2, rises slightly at P_H 7.0, exhibits the usual maximum at P_H 7.4, and then decreases with decrease in hydrogen ion concentration, the limiting concentration being beyond the culture testing P_H 10.0+.

Examination after incubation for 6 hours at 27° C. shows that no germination is evident at P_H 2.8, a fact noticed with the series incubated at 22° C. Slight germination occurs at P_H 3.1 and increases to the culture exhibiting the value P_H 5.0. Following the fall in germination at P_H 6.2, there occurs a slight rise at P_H 7.0 and the typical maximum at P_H 7.4. After this crest is

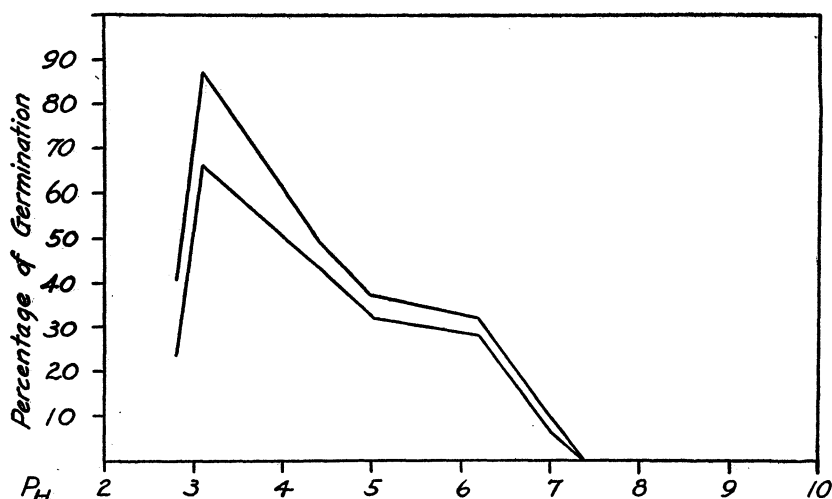


Fig. 5. *Lenzites saepiarum*. Graphic representation of the relation of germination to H ion concentration.

passed, germination decreases rapidly and appears to be totally inhibited at P_H 10.0+.

With an additional incubation of fourteen hours, the order of germination on the acid side shifts considerably, while that on the alkaline side remains practically the same. Slight germination is obtained at P_H 2.8, with the maximum on the acid side occurring at P_H 3.1, and these are the only significant changes. A series was also incubated at 31° C., but on finding that two of the solutions had become contaminated, the data were discarded. In fig. 4 are shown the germination curves for the successful temperatures with this organism.

Very uniform data are obtained with the spores of *Lenzites saepiarum*, as shown by table v.

TABLE V

LENZITES SAEPIARIA. AVERAGE PERCENTAGES OF SPORE GERMINATION IN M/5 MANNITE AT 25° C. AND AT VARIOUS HYDROGEN ION CONCENTRATIONS

Hrs.	Hydrogen ion concentration, P_H									
	2.8	3.1	4.4	5.0	6.2	7.0	7.4	8.8	9.6	10.0+
18	23.6	66.3	43.3	32.1	28.3	6.5	0.0	0.0	0.0	0.0
30	40.4	87.4	49.3	37.4	32.2	8.6	0.0	0.0	0.0	0.0

Minimum germination occurs in the culture testing P_H 7.0, and increases with increase of hydrogen ion concentration to the culture with the value P_H 3.1. At P_H 3.1 the maximum is obtained, and with further increase of hydrogen ions there is marked inhibition of germination. The results after incubation of 18 and 30 hours respectively, were very similar.

Due to limited time, it was possible to run only the one series. The curves in fig. 5 represent the germination at 25° C., one curve being constructed from the data after a period of incubation of 18 hours, the other after 30 hours.

Although no controls, as thought of in the usual sense, were run in the experiments reported in this paper, the cultures of each series possessing a hydrogen ion concentration of P_H 7.0 have been regarded as such, it being considered that mannite in doubly distilled water gives a solution with an approximately neutral reaction. Such cultures then contained H and OH ions in equilibrium together with the other ions common to the cultures of the entire series, namely, sodium and phosphorus.

DISCUSSION

It is believed that the results here presented are sufficient materially to change the prevailing view as to the relation of spore germination to acid and alkaline media. Among the forms studied, germination is a process which is strikingly supported by a relatively high hydrogen ion concentration. In certain forms, secondary maxima may occur at approximately the neutral point, but only in one case among those studied, *Fusarium* sp., is the primary maximum near the neutral point or on the alkaline side. It is not necessary, of course, to assume that the hydrogen ion concentration most favorable for germination will also prove most favorable for the continued growth and development of the organism. Moreover, that is a problem outside of the present investigation.

Inasmuch as ordinary nutrient media for pathological and bacteriological work usually exhibit a hydrogen ion concentration approximately neutral or slightly acid, the data obtained from this investigation are further interesting in that they show that successively increasing concentrations of hydrogen ions, from

neutrality, favorably influence germination of the spores of *Aspergillus niger*, *Penicillium cyclopium*, *Botrytis cinerea*, *Fusarium* sp., and *Lenzites saepiaria* up to approximately P_H 3.0. However, with increase of hydrogen ion concentration above this point, the germination quantities abruptly diminish. Some detailed discussion is however needed to compare these results with the work of others.

It has been shown that in the case of *Aspergillus niger*, maximum germination is obtained at P_H 3.1, which expressed in terms of normality is N/1259. At P_H 2.8, or N/631, germination is considerably better than at the neutral point, so that complete inhibition of germination must lie considerably higher than P_H 2.8. Since the foregoing hydrogen ion concentrations have been expressed in terms of normality, it might be well to cite the concentrations of certain acids allowing normal or almost normal development of the spores of *Aspergillus flavus* in beet decoction, as determined by Clark ('99): HCl, N/64; HNO₃, N/64; H₂SO₄, N/128; acetic, N/64; monochloroacetic, N/256; dichloroacetic, N/128; trichloroacetic, N/64; and HCN at N/8192. A mean of the limiting concentrations on the alkaline side for the various temperatures is P_H 8.6, or N/251200, from which it appears that OH ions have the greater toxicity. In beet decoction, Clark found that N/16 KOH injured the spores of *Aspergillus flavus*, while N/8 was fatal; also that N/32 NH₄OH inhibited germination, while N/16 was fatal. He concludes that the hydroxyl group, OH, is rather more toxic to the moulds studied than ionic H. However, as previously shown, the exact concentration of hydrogen ions in his cultures can not be calculated.

Penicillium cyclopium exhibits a relation to hydrogen ion concentration comparable with that of *Aspergillus niger*. Moreover, of all the forms which he studied, Clark found *Penicillium glaucum* the most resistant to acids and alkalis as well as to other poisons, the inhibiting concentrations on the whole being greater than those for *Aspergillus flavus*. Stevens' results indicated that *Penicillium crustaceum* is more resistant to poisons in aqueous solution than any of the other fungi studied by him. Growth occurred in N/50 HCl and H₂SO₄, while N/40 KOH and NaOH caused death. In my study, the rise in the germination

quantities at or about neutral is followed by a general decline. The limiting concentration on the alkaline side is about $P_H 10.0+$, thus presenting a range of germination greater than that of *Aspergillus niger*.

From my results, *Botrytis cinerea* may be regarded either as very sensitive to an alkaline reaction in mannite solution or else as manifesting a certain dependence upon the stimulating effects of hydrogen ion concentration under such conditions. Not only is germination inhibited at $P_H 7.0-7.4$, but the maximum, reached at $P_H 3.1-2.8$, is equivalent to about $N/1000$ acid. The range of germination in this case is small.

Lenzites saepiaria, like *Botrytis cinerea*, proved very sensitive to an alkaline reaction, and, while the limiting concentration of the former on the acid side is somewhat lower than the latter, the two fungi are similar in behavior. Meacham ('18) obtained inhibition of growth of *Lenzites saepiaria* at about $P_H 1.7$ in synthetic and malt-extract, and it is of interest to note that he frequently obtained a maximum of growth at about $P_H 3.0$, which approaches very closely the hydrogen ion concentration of $M/5$ mannite which affords maximum germination, as reported in this paper.

Of the forms studied, *Fusarium* sp. is the only one that responded favorably to an alkaline medium. Moreover, this form exhibits about the widest range of germination, yet the behavior was variable and discordant results were not infrequent.

CONCLUSIONS

Under the conditions described and as far as the experiments have gone, the following conclusions may be drawn:

(1) In a culture solution consisting of $M/5$ mannite, phosphoric acid, and sodium hydroxide, successively increasing concentrations of hydrogen ions from neutral or approximately neutral to $P_H 3.1-2.8$ favorably influence germination of the spores of *Aspergillus niger*, *Penicillium cyclopium*, *Botrytis cinerea*, *Fusarium* sp., and *Lenzites saepiaria*.

(2) The range of germination and the magnitude of the germination quantities as influenced by hydrogen ion concentration in the solution mentioned depend upon the organism, germina-

tion being obtained with the following concentrations, inclusive: *Aspergillus niger*, P_H 2.8–8.8; *Penicillium cyclopium*, 2.8–10.0+; *Botrytis cinerea*, 2.8–7.0; *Fusarium* sp., 2.8–10.0+; and *Lenzites saepiaria*, 2.8–7.0.

(3) It is not until a hydrogen ion concentration of P_H 2.8 or above is reached that inhibition of germination of the forms studied is noticed.

(4) *Aspergillus niger*, *Penicillium cyclopium*, *Botrytis cinerea*, and *Lenzites saepiaria* show a maximum of germination in the medium employed at P_H 2.8–3.1; *Fusarium* sp. exhibits a secondary maximum at this concentration.

(5) *Fusarium* sp. gives a pronounced maximum of germination at P_H 7.4, and *Penicillium cyclopium* exhibits a minor secondary maximum at P_H 7.0–7.4.

(6) For equal removes from the neutral point, OH ions appear to be relatively more toxic to the spores studied than H ions.

(7) With increase in length of intervals of incubation, the relations of germination to hydrogen ion concentration remain practically the same.

(8) The curves of germination for any organism are practically identical, whether incubated at 22° C., 27° C., or 31° C.

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